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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	DeBouck, *et al.*	11 July 2001
Serial No.:	09/297,701	Group Art Unit No.: 1655
Filed:	May 5, 1999	Examiner: Souaya, J.
For:	METHODS FOR IDENTIFYING GENES ESSENTIAL TO THE GROWTH OF AN ORGANISM	

Assistant Commissioner of Patents
Washington, D.C. 20231

AMENDMENT & RESPONSE UNDER 37 C.F.R. §1.111

In response to the Office Action mailed April 11, 2001 (Paper No. 9) (herein "Office Action"), the Applicants respectfully request entry into the record and consideration of this amendment and response. As this amendment and response is timely filed within the shortened statutory period for response of 3 months, no fee is required. Please charge any additional requisite fees relating to this amendment and response to Deposit Account No. 19-2570.

Please amend the above-identified application as follows:

In the Claims:

Please amend Claims 1 and 12 as follows:

1. (Twice Amended) A method of identifying genes essential to growth of a single celled organism comprising:

(a) preparing a genomic library of the single celled organism;

(b) providing a plurality of identical grids, each grid comprising a surface on which is immobilized at predefined regions on said surface a plurality of defined materials derived from the genomic library;

(c) mutagenizing the single celled organism by transfection with *(i)* a randomly integrated transposon or a similar insertional or transposable element of known sequence or *(ii)* with a constructed suicide vector;

(d) growing a test culture comprising the mutagenized single celled organisms and a control culture comprising non-mutagenized single celled organisms under a set of defined conditions;

(e) harvesting surviving cells from the cultures;

(f) extracting and isolating DNA from harvested cells of the test culture;

(g) extracting and isolating RNA or DNA from harvested cells of the control culture;

(h) generating labeled polynucleotide probes from the isolated DNA of the test culture using an oligonucleotide primer directed against *(i)* the randomly integrated transposon or similar insertional or transposable element of known sequence or *(ii)* the constructed suicide vector;

(i) generating labeled polynucleotide probes from the isolated RNA or DNA of the control culture;

(j) hybridizing the labeled probes generated from the isolated DNA of the test culture to a first identical grid to produce a test hybridization pattern;

(k) hybridizing the labeled probes generated from the isolated RNA or DNA of the control culture to a second identical grid to produce a control hybridization pattern;

(l) comparing the hybridization patterns to identify genes essential for growth in the single celled organism; and

(m) confirming that said identified gene is essential for growth of the single celled organism.

12. (Twice Amended) A method of identifying genes essential to growth of a single celled organism by identifying conditionally lethal mutant genes, which comprises:

(a) preparing a genomic library of the single celled organism: (*i*) in an integration vector; or (*ii*) in an expression vector;

(b) providing a grid comprising a surface on which is immobilized at predefined regions on said surface a plurality of defined materials derived from the genomic library;

(c) mutagenizing the single celled organism by transfection with *(i)* a randomly integrated transposon or a similar insertional or transposable element of known sequence or *(ii)* with a constructed suicide vector;

(d) growing the mutagenized single celled organisms under permissive and non-permissive conditions to identify mutagenized single celled organisms containing conditionally lethal mutant genes;

(e) transforming the single celled organism containing said conditionally lethal mutant genes with the genomic library of step (a);

(f) growing the transformed cells under the same non-permissive conditions as step (d) to identify transformed cells in which conditionally lethal mutant genes have been complemented;

(g) harvesting surviving cells;

(h) extracting and isolating DNA from the harvested cells;

(i) generating labeled polynucleotide probes from the isolated DNA using an oligonucleotide primer directed against *(i)* the randomly integrated transposon or similar insertion or transposable element of known sequence or *(ii)* the constructed suicide vector;

(j) hybridizing the labeled probes generated from the isolated DNA to a grid, whereby such probes that hybridize to the grid identify genes essential for growth of the single celled organism.

REMARKS

Claims 1-12 are pending in the instant application. Claims 1-12 stand rejected. No claims have been objected to. Claims 1 and 12 have been amended. In view of the Examiner's rejections of the Claims in the Office Action mailed April 11, 2001 (Paper No. 9), Applicants posit that the Examiner entered the preliminary amendment filed on January 4, 2001 along with

the request for Continued Prosecution Application (CPA) under 37 CFR 1.53(d). The Applicants respectfully request written confirmation indicating that the preliminary amendment was entered into the record. In view of the following amendment and response, the Applicants believe the claims presented herein are allowable. Reconsideration is respectfully requested.

Attached hereto as pages 11 and 12 is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

REJECTIONS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-12 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

At page 3, numbered paragraph 4 of the Office Action, the Examiner indicates that the use of the phrase "similar insertional..." in claims 1 and 11 is unclear as to what the claimed element is similar to. *Arguendo*, the Applicants presume the Examiner's rejections are intended for Claims 1 and 12 since the phrase "similar insertional..." is in Claim 12 and not Claim 11.

The Applicants respectfully disagree with the Examiner that there is a lack of clarity with respect to what claimed element is similar in Claims 1 and 12. The Applicants respectfully assert that the skilled artisan would recognize that randomly integrated transposons are just one class of insertional or transposable element of which there are many others known. Persons of ordinary skill in the art would have no difficulty in determining whether a given composition meets these criteria. Accordingly, the claims are definite within the meaning of § 112. *In re Mercier*, 185 U.S.P.Q. 774 (C.C.P.A. 1975) (claims sufficiently define an invention so long as one skilled in the art can determine what subject matter is or is not within the scope of the claims).

However, solely to facilitate prosecution, and in no way acquiescing to the Examiner's rejection, the Applicants have amended the claims to clarify the relationship between randomly integrated transposons and other insertional or transposable elements. No amendment made was for the purpose of narrowing the scope of any claim; but simply for clarity. The Applicants respectfully submit that in view of the forgoing remarks and the claims as amended, the Applicants have overcome the Examiner's rejection under 35 U.S.C. §112, second paragraph, and that rejection should be withdrawn.

REJECTIONS UNDER 35 U.S.C. §103(a)

Claims 1-12 are rejected under § 103(a) as allegedly being unpatentable over Bascomb *et al.* (EPO 0 680 722 A1) (herein "Bascomb") and Quandt, *et al.* (Gene, 1993, vol. 127, pp15-21) (herin "Quandt) in view of Lennon *et al.* (Trends in Genetics, October 1991, vol. 7, pp 314-317) (herein "Lennon"). Applicants respectfully traverse this rejection.

The Applicants assert that Bascomb and Quandt in view of Lennon would have provided the skilled artisan with neither the requisite motivation to carry out the modifications suggested by the Examiner nor the reasonable expectation of successfully arriving at the Applicants' claimed invention. Claims 1-12 are directed to methods for identifying genes essential to the growth of an organism using a grid prepared from a genomic library of single celled organisms. The Examiner impermissibly uses hindsight reconstruction with Bascomb and Quandt in view of Lennon to make this *prima facie* obviousness rejection of the instant claims 1-12 and has met neither prong of burden required by *In re Vaek, supra* for the following reasons.

Bascomb's teaching is limited to a screening method to identify herbicides that target known or classified, essential enzymatic pathways from plants. These enzymes or metabolic pathways are specific to plants and require genetic complementation of a microbial organism, *i.e.*

bacterial or fungal organisms. The identification of these pathway-specific herbicides are dependent on compounds that inhibit the function of known essential enzymes, *i.e.* proteins. In contrast, Lennon's method relies on arrayed cDNA (made from mRNA) libraries that are probed with labeled cDNA to determine differential expression between conditions. Applicants respectfully disagree with the Examiner's allegation that using the method of screening of Lennon to perform the method of Bascomb would have made the screening method of Bascomb easier to perform because Bascomb relies on enzymatic function and Lennon relies on RNA transcripts.

In addition, Applicants assert that a mutation of an essential plant gene obtained using the site-directed gene replacement experiment of Quandt could transcribe an RNA fragment from the mutated gene that would fail to result in a functional protein, *i.e.* the gene can still be expressed. Therefore, the RNA produced would be converted to cDNA and hybridized to the Lennon cDNA array. The resulting mutant hybridization pattern would be identical to the wild-type hybridization pattern for that gene. Bascomb and Quandt in view of Lennon neither teach nor suggest using extracted DNA of a bacterial, or any other test culture as templates in primer extension reactions where the oligonucleotide primers are directed against the inserted elements and the reaction extends into the flanking DNA sequence (see page 10 of the instant application, starting at line 10). By start contrast, mutant genes containing randomly integrated transposon or a similar insertional or transposable element of known sequence or a constructed suicide vector will generate probes that will hybridize with grids of the instant invention.

The Office Action points to nothing in the cited references that would impel a modification of the disclosed methods necessary to arrive at the instant invention. The Examiner alleges that the claimed invention would have been obvious because it would have been possible to combine site-directed suicide vector gene replacement experiments that eliminate long and tedious screening procedures culled from Quandt and modify Bascomb's herbicide screen in a

way which could then be modified to screen for essential genes in microorganisms in view of modifying Lennon's cDNA expression arrays to screen for herbicides that inhibit enzymatic pathways, i.e. proteins. However, even if one skilled in the art could combine the above mentioned modifications it would not result in the instant invention. Moreover, the mere possibility that the prior art can be combined and modified does not itself provide the requisite motivation to do so. *In re Dien*, 152 U.S.P.Q. 550 (C.C.P.A. 1967) (incentive to seek improvement of existing process held to not render change made by applicant obvious, even where the change was one capable of being made from theoretical point of view). The mere possibility for modification and improvement is not the "motivating force" that the Patent Office Board of Appeals and the Federal Circuit have invariably required. If it were, then no modification would ever lack motivation since some change is always possible. Quite to the contrary, an invention is obvious under the patent laws only when the claimed means for effecting an improvement -- as opposed to the possibility of trying any and all means -- is suggested by the prior art. *In re Shaffer*, 108 U.S.P.Q. 326 (C.C.P.A. 1956) (references, viewed by themselves and not in retrospect, must suggest doing what applicant has done). Significantly, neither of the cited references would have motivated persons of ordinary skill to make the substantial modifications that would have been necessary to produce the claimed invention. It is only with the improper use of hindsight and with the benefit of the Applicants' disclosure that one can discern the desirability of the particular invention now claimed.

In summary, the Applicants' invention is identifying genes essential for growth of a single celled organism. Bascomb's method is identifying herbicides that inhibit plant protein function. Bascomb and Quandt are methods that both require the known sequence of the genes involved. Lennon's technology would neither simplify nor suggest the present invention because Bascomb relies on protein function and Lennon relies on RNA transcription.

Neither Bascomb nor Quandt, disclose or suggest a method of identifying genes essential to the growth of single celled organisms. The secondary reference of Lennon alone or in combination, fail to cure these deficiencies of the primary references.

The Examiner has also rejected Claims 1-12 as being unpatentable over Nishi *et al.* (JBC, March 1994, vol. 269, pp6320-6324) (herein "Nishi") in view of Lennon. *Arguendo*, the Applicants posit that the Examiner's rejections on page 6, numbered paragraph 7 of the Office Action are under § 103(a) as allegedly being unpatentable over Nishi and Quandt in view of Lennon. The basis for the Applicants' position is the use of Nishi and Quandt in the Examiner's rejection on page 7.

The skilled artisan would not use Nishi and Quandt's methods in view of Lennon to arrive at the Applicants' claimed gridding method. If genomic DNA is extracted from the wild-type LMB sensitive strain (JY266), and mutant LMB resistant strain (LM102), and wild-type *S. pombe* genomic DNA is used as template for two separate hybridization probes, there would be no way of identifying the difference between the two hybridization patterns. The genomic grids would not be usable to distinguish between the dominant (LMB resistant) form of the *crm*1 gene and the recessive (LMB sensitive) form of the *crm*1 gene. The "genomic DNA" labeled and hybridized to the "genomic grids" would result in both *S. pombe* strains showing identical hybridization patterns due to slight mutations in the essential gene sequences that likely would not interfere with DNA-DNA hybridizations. In fact, the whole genome will likely hybridize to the grid forming no patterns in either case.

Nishi and Quandt in view of Lennon neither teach nor suggest using extracted DNA of the test culture as templates in primer extension reactions where the oligonucleotide primers are directed against the inserted elements and the reaction extends into the flanking DNA sequence.

Only mutant genes containing a randomly integrated transposon or a similar insertional or transposable element of known sequence or a constructed suicide vector will generate probe that will hybridize with the grids.

By contrast, Lennon merely teaches a method of screening that involves generating a plurality of filters to form a grid, and each grid contains immobilized cDNA clones at predefined regions (pages 314, col. 2, first para; page 315, col. 1 last para and col. 2). The Examiner points out that Lennon suggests the use of a "genomic" cDNA library (page 314, col. 2, last paragraph). The context of Lennon's use of the word "genomic" does not render the present invention obvious. Specifically, Lennon states that "[w]hile cDNA libraries are usually made from cytoplasmic poly(A)$^+$ RNA, it might also behoove the genome community to generate cDNA libraries from poly(A)$^-$ brain RNA, for estimates have indicated that there could have indicated that there could be close to 18,000 genes expressed in postnatal brain uniquely as poly (A)$^-$ transcripts; if these are *bona fide* genes, most are probably brain specific, and certain neurological disorders may be caused by mutations in such genes" (pages 314, col. 2, last paragraph). Lennon refers to a "genomic" cDNA library as a library containing cDNA made from poly(A)$^+$ and poly (A)$^-$ RNAs. However, Lennon does not teach genomic DNA perse. Therefore, Lennon's screen along with the teachings of Bascomb and Quandt or Nishi and Quandt are not obvious because Lennon's methods require the isolation of expressed RNA to produce cDNA. cDNA is not a representation of the genome of an organism. In fact, this "genomic" cDNA library screen will be limited to only expressed genes in the brain.

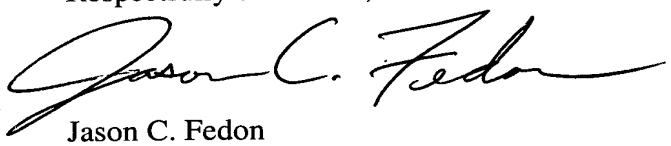
Neither Nishi nor Quandt, disclose or suggest a method of identifying genes essential to the growth of single celled organisms. The secondary reference of Lennon alone or in combination, fail to cure these deficiencies of the primary references.

In view of the forgoing remarks, the Applicants respectfully submit that they have overcome all grounds of the Examiner's rejections based on 35 USC §103(a), first paragraph, and respectfully request that this rejection be withdrawn.

The Applicants reserve the right to prosecute, in one or more patent applications, the claims to non-elected inventions, the claims as originally filed, and any other claims supported by the specification. The Applicants thank the Examiner for the Office Action and believe this response to be a full and complete response to such Office Action. Accordingly, favorable reconsideration and allowance of the pending claims is earnestly solicited.

If it would expedite the prosecution of this application, the Examiner is invited to confer with the Applicants' undersigned agent.

Respectfully submitted,



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